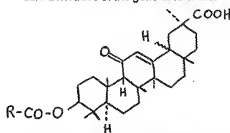


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 GB 1475075  
 GB 1387499  
 GB 1255672  
 GB 1046566  
 GB 1032710  
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 GB 843133  
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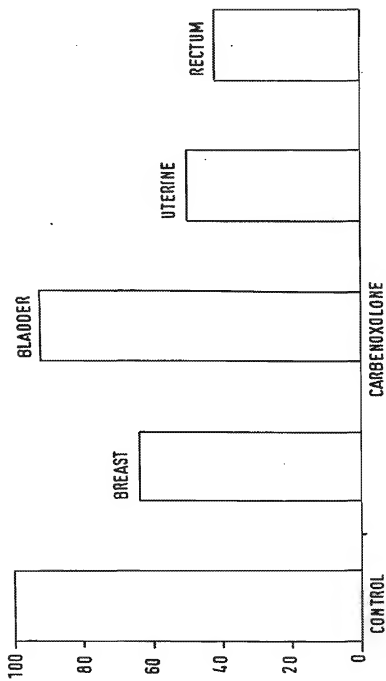
(54) Anti-neoplastic compositions containing esterified glycyrrhetic acid

(57) Pharmaceutical compositions for treating, alleviating and ameliorating the symptoms of cancer in humans, comprise at least one glycyrrhetic acid derivative of the general formula:-



tic radical, which optionally contains one or more carboxylic groups, and/or at least one pharmaceutically compatible salt thereof, in admixture with a pharmaceutical diluent or carrier. The compositions may be in forms suitable for oral, rectal, vaginal or parenteral administration.

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EFFECT OF CARBENOXOLONE ON  
TUMOUR CELL CULTURES.%. RATE OF  $H^3$  THYMIDINE INCORPORATION  
AS COMPARED WITH CONTROLS.

## SPECIFICATION

## Cytostatic compositions

5 The present invention is concerned with novel cytostatic compositions.

For many years, investigations concerned with tests to determine sensitivity of leukaemias to cytostatic agents have been successfully carried out and have found clinical application. However, similar research with regard to solid tumours has been less effective. Either it was necessary to process the tumour tissues and produce single cell-layer cultures, which never permit definite conclusions with regard to the

10 metabolism of the original tissues, or metabolic parameters and their reactions to cytostatic agents are determined on tissue particles, which are not relevant to the question of sensitivity to cytostatic agents. It must also be taken into account that, for *in vitro* tests, it is essential, in principle, that the cytostatic mechanisms of action of the chemotherapeutic agents under investigation are largely known. The inhibitory actions with cytostatic effect of a drug, with reference to the metabolic parameters under assessment, must

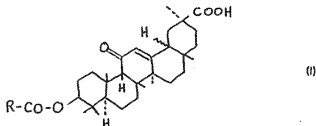
15 have been investigated beforehand. One particular finding has been that, when the nucleic acid metabolism of tumour tissue is studied by using radio-active precursors, there is no inhibition of incorporation of one single precursor, which is of crucial significance for all *in vitro* test results with different cytostatic agents.

Investigations have been carried out to determine *in vitro* the rates of incorporation of various  $H^3$ -labelled precursors of deoxyribonucleic acid (DNA) into the nucleic acid of solid malignant

20 tumours and to determine the effect of inhibition of different cytostatic agents on the parameters studied. The experiments were done in order to ascertain whether it is possible, with such determinations in the nucleic acid metabolism of tissues particles, to assess the sensitivity of solid tumours to cytostatic agents and in that way to gain orientation for effective combined surgical and chemotherapeutic treatment of malignant tumours.

25 As a result of our investigations, we have now found that glycyrrhetic acid which is esterified in the 3-position with a mono-, di- or polybasic acid exhibits a remarkable activity in inhibiting neoplastic growths and is, therefore, a valuable cytostatic agent.

The active compounds in question can be represented by the general formula:-



40 wherein R is an aliphatic or cycloaliphatic radical, which optionally contains one or more carboxylic groups; and the pharmaceutically compatible salts thereof.

The compounds of general formula (I) are known and several of them have already been used topically externally and parenterally for the treatment of inflammatory and ulcerative diseases.

Compounds of general formula (I) and salts thereof have, for example, been described and claimed in our

45 British Patent Specifications Nos. 843,133; 950,777; 1,022,968; 1,032,710 and 1,387,499.

The compounds used according to the present invention can be mixed in the usual manner with appropriate pharmaceutical diluents or carriers, aroma, flavouring and colouring materials and formed, for example, in tablets or dragees or, with the addition of appropriate adjuvants, suspended or dissolved in water or in an oil, for example olive oil. The compounds can also be in lyophilised or micronised form and

50 mixed with an appropriate liquid carrier or diluent immediately prior to administration.

The compounds used according to the present invention can be administered orally or parenterally in admixture with solid or liquid pharmaceutical diluents or carriers. As injection medium, it is preferred to use water which contains the stabilising agents, solubilising agents and/or buffers conventionally used in injection solutions. Injection solutions must, of course, be sterile. Additives of this kind include, for example,

55 tartrate, citrate and borate buffers, ethanol, dimethyl sulphoxide, complex-forming agents (such as ethylene-diamine-tetraacetic acid and the non-toxic salts thereof) and high molecular weight polymers (such as liquid polyethylene oxide) for viscosity regulation.

Solid carrier materials include, for example, starch, lactose, mannitol, methyl cellulose, highly-dispersed silicic acid, gelatine, agar-agar, calcium phosphate, magnesium stearate, animal and vegetable fats and solid

60 high molecular weight polymers (such as polyethylene glycols). Compositions suitable for oral administration can, if desired, contain flavouring and/or sweetening agents.

For the treatment of neoplasms and especially of various forms of cancer, the active compounds can be administered orally or rectally for the treatment of neoplastic diseases of the gastro-intestinal tract, or vaginally for the treatment of neoplastic diseases of the vagina or uterus or by injection for the treatment of,

65 for example, neoplastic diseases of the mammary glands.

Many antineoplastic agents which have previously been used suffer from the disadvantage of producing distressing side-effects, such as disturbances of the gastro-intestinal tract, stomatitis, vomiting, skin reactions, alopecia, hematopoietic depression and neuropathy. The active materials used according to the present invention do not give rise to any of these highly undesirable side-effects; indeed, some of these active materials have already been extensively used in clinical practice for the treatment of gastro-intestinal disturbances and the like.

In order to demonstrate the effectiveness of the active materials to be used according to the present invention, one of them, namely the disodium salt of glycyrrhetic acid hemisuccinate ( $R = \text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}_2$ ) (also known as carbenoxolone sodium) has been tested *in vitro* against several malignant solid tumours and has been compared with three known antineoplastic agents, namely, vincristine, methotrexate and 5-fluorouracil. The effectiveness of the test compounds was determined from the rates of incorporation of  $\text{H}^3$ -labelled thymidine (measured as impulses per minute (imp)) into malignant solid tumours.

The test procedure employed is as follows:

- A cell suspension prepared from tumours immediately after surgical removal, is put through a strainer and suspended in buffered glucose solution (Krebs-Ringer phosphate buffer; pH 7.36; glucose 300 mg%) at  $0^\circ\text{C}$  and the supernatant poured off. The cells are then suspended in an incubation medium. After staining with nigrosine, the proportion of dead tissue particles is determined under the microscope; 1 ml. amounts of this suspension are pipetted into centrifuge glasses containing, with the exception of controls, the cytostatic agents under investigation dissolved in 0.1 ml. of buffer (amount calculated to give final concentration).
- Preliminary incubation of the tissue particles with the different cytostatic agents is for 30 minutes at  $37^\circ\text{C}$  in a shaking water-bath. The labelling substance, for example  $\text{H}^3$ -labelled thymidine (6.2 Ci/mMol) is then added in an ice-bath in an amount of 0.1 Ci per glass and per assay. Each tube in an assay received only one labelling substance in order to determine the rate of incorporation of that substance. Labelling proceeded in a shaking water-bath at  $37^\circ\text{C}$ . for 60 minutes so that the total duration of incubation is 90 minutes. The pH of the assays is checked during incubation and kept at pH 7.3. At a constant pH, the tissue particles show linear incorporation rates of the labelled substances during 2 hours under these experimental conditions.

The rates of incorporation of assays treated with cytostatic agents are related to the untreated controls, the incorporation in the latter being taken as being equal to 100%.

- The results obtained are given in the following Table 1, these results being obtained from 3 experiments on a human oral carcinoma cell line. These experiments clearly show that carbenoxolone sodium depresses DNA synthesis to an extent of at least 70%, compared with the controls. The Figure of the accompanying drawing shows the results obtained with the carbenoxolone sodium on four different tumours.

TABLE 1  
Rate of  $H^3$  thymidine incorporation into HEP<sub>m</sub> cells in culture and effect of cytostatic agents and of carbenoxolone on this incorporation

| Cytostatic Agents    | Experiment 1<br>N = 3 |                     |  | Experiment 2<br>N = 3 |                     |  | Experiment 3<br>N = 3 |                     |  |
|----------------------|-----------------------|---------------------|--|-----------------------|---------------------|--|-----------------------|---------------------|--|
|                      | DPM $\pm$ SD          | Change %<br>Control |  | DPM $\pm$ SD          | Change %<br>Control |  | DPM $\pm$ SD          | Change %<br>Control |  |
| Control              | 58,400 $\pm$ 22,100   | 100                 |  | 24,020 $\pm$ 1,900    | 100                 |  | 38,790 $\pm$ 7,400    | 100                 |  |
| Vincristine          | 15,160 $\pm$ 2,690    | 26                  |  | 16,670 $\pm$ 2,090    | 69                  |  | 9,080 $\pm$ 380       | 23                  |  |
| Methotrexate         | 19,400 $\pm$ 4,440    | 33                  |  | 24,450 $\pm$ 3,540    | 102                 |  | 32,810 $\pm$ 1,610    | 82                  |  |
| 5-Fluorouracil       | 31,300 $\pm$ 6,382    | 54                  |  | 19,690 $\pm$ 190      | 82                  |  | 17,420 $\pm$ 10,440   | 44                  |  |
| Carbenoxolone sodium | 2,340 $\pm$ 910       | 4                   |  | 8,760 $\pm$ 1,304     | 36                  |  | 2,930 $\pm$ 1,800     | 7                   |  |

The results obtained clearly show that carbenoxolone sodium is much more effective than methotrexate, vincristine and 5-fluorouracil and also shows that carbenoxolone sodium is very effective in rectal and uterine cancers and is moderately effective in mammary and bladder cancers.

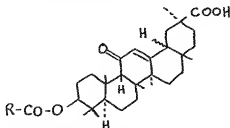
Recent clinical trials have demonstrated the effectiveness of the active compounds of general formula (I) in prolonging the life of terminal cancer patients. In some cases, life has been prolonged for several months, coupled with a remarkable regression of the side effects associated with terminal cancer, including anorexia and dyspepsia. Furthermore, the quality of life of the treated patients showed a remarkable improvement. The following Table 2 summarises some of the clinical results which have been obtained with the use of carbenoxolone sodium. In all cases, the patients were terminal. As can be seen, in many cases the treatment resulted in survival being prolonged for several months, during which time there was also a remarkable improvement in the quality of life of the patients.

Table 2

| Age | Carbenoxolone dosage/day In mg | Length of treatment | Complications   | Clinical observations during treatment  | Survival       | Cause of death                |
|-----|--------------------------------|---------------------|---|---|----------------|-------------------------------|
| 56  | 200 - 100                      | 4 months            | Hypokalaemia  | Symptom free until oedema developed at 4 months                                   | 4 months       | Carcinomatosis                |
| 71  | 500                            | 7 months            | None  | Reasonably well   | 7 months       | Carcinomatosis                |
| 60  | 500                            | 3 months            | None  | Progressive anorexia and weight loss  | 3 months       | Carcinomatosis                |
| 60  | 500 tumour injected            | 2 months            | Hypokalaemia  | Shrinkage of tumour, improvement of dyspepsia, development of cerebral metastases | 2 months       | Cerebral haemorrhage          |
| 53  | 500                            | 1 month             | Hypokalaemia  | Persistent dyspepsia  | 4 months       | Carcinomatosis                |
| 75  | 500                            | 10 days             | Hypokalaemia<br>Nausea, anorexia<br>Patient refused potassium supplements |   | No follow-up   |                               |
| 51  | 500                            | 8 weeks             | Hypokalaemia  | Dyspepsia improved  | 2 months       | Carcinomatosis                |
| 75  | 500                            | 12 months           | None  | Considerable improvement in dyspepsia and anorexia                                | Alive and well |                               |
| 58  | 500                            | 2 weeks             | Hypokalaemia  | Dyspepsia improved  | 3 months       | Carcinomatosis                |
| 60  | 500                            | 6 months            | None  | Symptomatically well for 6 months   | 6 months       | Carcinomatosis                |
| 75  | 500                            | 10 days             | None  | Increasing jaundice and fluid retention   | 10 days        | Liver failure, carcinomatosis |
| 69  | 500                            | 2 weeks             | None  | Jaundice and fluid retention  | 3 weeks        | Liver failure, carcinomatosis |
| 82  | 500 tumour injected            | 2 weeks             | None  | Progressive dysphagia and anorexia  | 3 weeks        | Carcinomatosis, perforated    |

## CLAIMS

1. A composition for treating, alleviating and ameliorating the symptoms of cancer of humans, which comprises at least one active compound of the general formula:-



5 wherein R is an aliphatic or cycloaliphatic radical, which optionally contains one or more carboxylic groups, and/or at least one pharmaceutically compatible salt thereof, in admixture with a pharmaceutical diluent or carrier.

2. A composition according to claim 1, wherein the composition is in lyophilised form.

3. A composition according to claim 1, wherein the composition is in micronised form.

10 4. A composition according to claim 1, in the form of a sterile injectable solution.

5. A composition according to any of the preceding claims, in the form of a dosage unit containing an effective amount of the active compound.

6. A composition according to claim 1, substantially as hereinbefore described and exemplified.